Effects of *Lactobacillus plantarum* Administration in Peste Des Petits Ruminants Vaccinated Red Sokoto Goats: Changes in Nasal Bacterial Flora, Bronchoalveolar Cells and Lung Pathology

Tenuche Zaynab Oremeyi[1*] Emikpe Benjamin Obukowho[2], Egwu Godwin Onyeamaechi[3] and Taiwo Victor[4]

[1]*Department of Veterinary Pathology, University of Abuja, Abuja, Nigeria*

[2]*Department of Veterinary Pathology, Kwame Nkrumah University of Science and Technology, Khumasi, Ghana*

[3]*Department of Veterinary Medicine, University of Abuja, Abuja, Nigeria*

[4]*Department of Veterinary Pathology University of Ibadan, Oyo State Nigeria*

Corresponding Author*

Tenuche Zaynab Oremeyi
Department of Veterinary Pathology,
University of Abuja,
Abuja, Nigeria,
E-mail: oremeyiztenuche@gmail.com

Received: December 01, 2022, Manuscript No. JMBI-22-82026; **Editor assigned:** December 05, 2022, PreQC No. JMBI-22-82026 (PQ); **Reviewed:** December 19, 2022, QC No. JMBI-22-82026; **Revised:** February 23, 2022, Manuscript No. JMBI-22-82026 (R); **Published:** March 02, 2023, DOI: 10.4172/ JMBI.5.1.001

Abstract

Goats play an important role in the livelihood of the rural farmer usually involved in rearing them. They are reported to be highly prolific, hardy and serve as a source of food security (providing a good source of animal protein from their meat and milk) and income (through sale of live animals, carcasses or by product) helping to make ends meet. In Nigeria, the red sokoto goat is a breed predominantly found in the northern region of the country and constitutes 60% of the total goat population.

Pneumonia remains a major constraint in goat farming. Peste des petits ruminants and *Mannheimia haemolytica* are two pathogens that have been implicated in field outbreaks of caprine pneumonia in Nigeria which account for high morbidity, reduced productivity, wasting of carcasses and high mortality, leading to huge economic losses for the farmers.

Keywords: *Mannheimia haemolytica* • High morbidity • Reduced productivity • High mortality

Introduction

The control of naturally occurring pneumonia as it exists in Nigeria is largely dependent on the PPR N75/1 vaccine which only caters to the viral pathogenic cause of pneumonia while the bacterial co-infection is only managed therapeutically with the use of antibiotics like Oxytetracyclin, Penicillin, Gentamycin and Enrofloxacin. However, the resistance of *M. haemolytica* to some of these antibiotics have been reported [1]. The need to explore alternative measures in the control of *M. haemolytica* becomes more urgent as bacterins produced in the past have failed to protect Nigerian goats from *M. haemolytica* pneumonia or inadvertently exposed the vaccinated animals to infection caused by the pathogen [2-4].

The consideration to explore an alternative in the control of natural occurring caprine pneumonia using a probiotic becomes pertinent. A probiotic is defined as "a live microorganism which confers health benefits on their host when given in adequate amounts" [5]. A probiotic like *Lactobacillus plantarum* have been shown to possess immunomodulatory effects in humans, reduce gut bacterial colonization as well as viral infectivity in pigs, and improve gut-health in ruminants [6].

Largely, the oral administration of *L. plantarum* has shown potential benefits to the respiratory tract through the gut-lung synergy [7]. However, obvious limitations include the fact that quite a number of these organisms are destroyed in the rumen during passage [8]. Hence, the need to investigate an alternative intranasal route of administration of *L. plantarum* (Probiotic) on the nasal bacterial flora and evaluate its efficacy in enhancing local immune responses in the respiratory in the upper respiratory tract in red sokoto goats.

Literature Review

Study area

The experimental animals and laboratory analysis of samples collected were carried out respectively at the experimental animal unit and diagnostic laboratory of the veterinary teaching hospital, faculty of veterinary medicine, university of Abuja.

Experimental animals

Each goat was obtained from recognised breeders within Abuja, F.C.T. A total of 20 Red Sokoto goats (female, n=8; male, n=12) were used in this study. They were between 6 months to 1 year of age and weighed an average of 8 kg.

The goats were randomly divided into four groups A,B,C and D with 5 animals in each group. Each group was housed in flea proof well partitioned pens with concrete floors. Acclimatization lasted for a period of two weeks before the experiment commenced. At this time, the animals were tagged to ease identification, and pre-treated with 20% Oxytetracyclin (L.A) at 1 ml/10 kg stat; Ivermectin 1 ml/50 kg subcutaneous injection, and vitamins at 10 mg/L of water was administered to alleviate stress for 5 days. Wheat bran/grass was provided twice daily as feed, while clean drinking water was given ad libitum. Adequate measures to minimize discomfort or pain to the animals were taken in the course of this experiment [9].

Vaccination

All experimental animals were vaccinated using PPR vaccine (N75/1). The recommended dosage of 1 ml (2.5 TCID50) was administered to each animal subcutaneously.

Lactobacillus plantarum: Source, dosage and intranasal inoculation.

The *L. plantarum* was obtained from the department of microbiology, university of Ibadan. Intranasal infusion of *L. plantarum* 1 ml (3.4×10^7) was carried out in goats in groups A, B and C for: Three, (group A); five (group B) and seven (group C) days consecutively. Goats in group D were infused intranasally with normal saline (1 ml).

Intranasal inoculation was done using a 1 litre hand held pressure sprayer as described by Ezeasor C, et al. Briefly, the handle was pumped severally until resistance was observed to build some pressure inside the bottle where the *L. plantarum* in MRS broth had been introduced. Each goat was then restrained with the head tilted backward slightly, and outer nares were cleaned using cotton wool soaked in water. One nostril was obstructed with the thumb, while the nozzle of the spray bottle was inserted into the other and a button was pushed to release a mist into the nostril. The action was repeated in the second nostril [10].

Bacteriology: Nasal swabs were collected once weekly, for four weeks (duration of study).

Sample collection: Nasal swabs were collected aseptically from each tagged goat after thorough cleaning of the external nares with a disinfectant. Collection was done by introducing sterile applicator sticks with cotton tip into each nostril and gently swirling around to get a representative bacterial sample from each nostril. The swab sticks were then dipped into bijou bottles containing 2 mls of BHI broth and the ends of the sticks were carefully cut and bottle caps were closed and tightened. The samples were transported in ice packs to the bacteriology laboratory of the microbiology department, university of Ibadan for bacterial isolation.

Culture media

Nutrient agar and sheep blood agar (Oxoid™) was the general all purpose media while brain heart infusion broth (Oxoid™) served as the transport medium. Differential and selective media (e.g. Baird parker agar, MacConkey agar, Eosin Methylene Blue (EMB) agar, Edward's medium, and sheep blood agar) for the species identification and enumeration (*Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* spp*., Pasteurella mulocida* and *Mannheimia haemolytica*) and prepared according to manufacturer's specification.

Bacterial culture

Swabs from the net sample were inoculated onto all purpose media (nutrient agar and sheep blood agar) and incubated for 24 hours at 37℃.

Enumeration of selected bacterial species

The bacterial cells were suspended and homogenised using BHI broth in tenfold serial dilution. 0.1 ml of each dilution was seeded onto each of the five sections drawn on a petri dish in the different selective and differential agar. The inoculated plates were then incubated at 37℃ for 24 hours. Following incubation, plates with the highest dilution with colonies of between 30 and 300 were enumerated as colony forming units (cfu) per ml, *i.e.* (Nos of colonies × dilution × dilution factor). Colonies were identified based on morphology and gram staining.

Bacterial identification biochemical characterization

Representative colonies were further subjected enzymatic (e.g. indole, ornithine decarboxylase, citrate utilization test, hydrogen sulphide, methyl red, vogues-proskauer test) and biochemical tests for characterisation in peptone water base using various sugars (fructose, glucose, mannitol, sucrose, lactose, galactose, maltose) as described earlier.

Isolation and identification of selected bacterial organisms of the nasal flora

Gram negative bacteria and mixed colonies were sub-cultured on blood and MacConkey agar at for 24 hours at 37℃. Isolates obtained were then sub-cultured into other prepared culture media to get pure colonies from the mixed colonies on the plates. Characterization and identification of the isolates were carried out using standard methods [11].

Bacterial count

The bacterial colony counts were performed following standard methods as described by Miles, et al., as modified by Herigstad, et al. The bacterial cells were suspended and homogenised using BHI broth in tenfold serial dilution. 0.1 ml of each dilution was seeded onto one of six sections drawn on a petri dish containing agar. The agar plates were then incubated at 37℃ for 24 hrs-48 hrs, after which colonies were counted. For each bacterial species, only sections with counts of 30 cfu-300 cfu were selected for consideration and recorded.

Necropsy and histopathology

During gross examination, changes in lung morphology were emphasized. Physical inspection of lung lobes following its exteriorization from the thoracic cavity was done by evaluating the degree of exudation, adhesions, and foci of consolidation. Changes in texture/consistency were determined by palpation. Other pathological features like gray/red hepatisation, necrosis, or haemorrhages were recorded as described by Lopez. Lung morphometry was also determined as described by emikpe and akpavie. Tissue sections from affected lobes were preserved in 10%Formalin for tissue processing and histopathology. For histopathology, tissue was processed as described by Akpavie. Sections were stained using Hematoxylin and Eosin, and viewed under the light microscope [12,13].

Statistical analysis

Mean and standard deviation were used to describe the data while Analysis of Variance (ANOVA) was used to compare the mean of various parameters considered in the study. Least Significant Difference (LSD) and Duncan Multiple Range Test (DMRT) were used as post hoc to ascertain significant difference between groups.

Results

Microbiology

The effects of the intranasal administration of *L. plantarum* (iLp) on selected bacteria of the upper respiratory micro flora showed significant reduction of *M. haemolytica* ($P \leq 0.05$) in all the iLp treated groups (A-iLp 3x, B-iLp 5x, and C-iLp 7x) irrespective of frequency of administration compared to the control group (D-intranasal normal saline). This decrease in bacterial count was also recorded for *Staphylococcus aureus* and *Escherichia coli* ($P \leq 0.05$). The iLp treatment however had no effect on the total bacteria count in the treatment groups. *L. plantarum* counts remained high throughout the course of the experiment in the treatment groups A, B, and C.

Pathology

Minimal to no pathology was observed in all the experimental animals of the iLp treated groups irrespective of the frequency of administration. Mild foci of consolidation were observed on the posterior cranial lobe (0.75%) and caudal lobe (0.40%) of the right lung of two goats in group A. In group C (iLp 7x) only one goat showed mild consolidation of the right anterior cranial lobe (0.70%). Also, in the control group D (iNS), mild consolidation of either the posterior cranial, accessory or caudal lobe of the left lung were observed in three of the experimental animals. Moderate congestion of the caudal lobes of the lung was also observed in one goat. All the experimental animals in group B (iLp 5x) showed no significant gross changes of their lung lobes [14].

Average lung consolidation score: The average lung consolidation scores were generally low across groups with the highest average consolidation score of 0.5% recorded in the iLp untreated group (D-iNS). The iLp treated groups (A, B and C) had lower lung consolidation scores. The least consolidation score (0%) was recorded in group B (iLp 5x). For groups A (iLp 3x) and C (iLp 5x) where a mild consolidation of different portions of the right cranial lobes, and left caudal lobe were observed, average lung consolidation scores of 0.23% and 0.14% were recorded respectively.

BALF cellular analysis: Generally, the backgrounds of the smears were clear and the cells occurred singly. High macrophage counts were recorded across all the groups, with groups B, A and C (iLp treatment groups) having the higher counts compared to group D (iNS). Also it was observed that although generally the lymphocyte and neutrophil counts were lower than the macrophage count, lymphocyte count was higher than neutrophil counts in the iLp treated groups (A, B and C) but lower than the neutrophil count in group D.

The macrophage: Neutrophil ratio (M:N) was highest in group B (iLP 5x-23:1), followed by group A (iLp 3x-15:1) then group C (iLP 7x-13:1) while group D (iNS 5:1) had the least ratio recorded [15].

Histopathology

There were no significant histopathological changes observed across the different groups (iLp treated or untreated). However, a remarkable change observed in the iLp treated groups A-3x, B-5x and C-7x was the marked infiltration by mononuclear cells (Bronchus Associated Lymphoid Tissue (BALT)) forming nodules of varying sizes at the basal region of the bronchial epithelium (plate 1; A-D). The peripheral area of the nodule were made up of predominantly lymphocytes and stained deeply basophilic, while the lighter staining center consisted also of macrophages, lymphocytes and very few plasma cells. High endothelial venules were also present adjacent and within these lymphocytic follicles. On the other hand, animals in group D (iNS), showed a mild aggregation of mononuclear cells (lymphocytes) (plate 1; D). Consequently, higher numbers of BALT were observed in the iLp treated groups than the control, with the highest nodular formation of BALT recorded in group B (iLp 5x), followed by C (iLp 7x) and A (iLp 3x) respectively. The sizes of the BALT was highest in group B (iLp 5x; 442028.4 ± 6232.36 cm^2), and generally higher in the iLp treated groups A (iLp 3x; 123592.8 ± 19458.02 cm^2) and C (iLp 7x; 319551.2 ± 15365.72 cm^2) compared to the control group D (iNS; 22017.6 ± 4332.72 cm^2).

Discussion

Although peste des petits ruminant and *Mannheimia haemolytica* have been commonly implicated in naturally occurring caprine pneumonia in this environment, misdiagnosis of the aetiologic agent during outbreaks of pneumonia has led to the rampant use of different non-specific antibiotics. This has led to widely reported antibiotic resistance amongst pathogenic strains of *M. haemolytica*. Also another side effect in the use of antibiotics is the disruption of bacterial homeostasis of normal flora following treatment. Therefore, this study was designed to evaluate the effect of intranasal administration of *L. plantarum* on the upper respiratory micro flora and pulmonary gross and microscopic morphology in Red Sokoto goats [16].

The results showed that in the iLp treated groups (A- iLp 3x; B-iLp 5x and C-iLp 7x) there was a significant decline in a few selected potentially pathogenic organisms of the upper respiratory micro flora (*M. haemolytica, S. aureus* and *E.coli*) counts studied especially in groups where the probiotic was administered for 5 and 7 consecutive days compared to the control group D (iNS). Also *L. plantarum* continued to be present in the UR micro flora throughout the period of this study. These findings illustrates not only, the competence of this probiotic to favourably displace these pathogens, probably by producing bacteriocidal compounds to eliminate them, but also sustaining its replicative ability in the UR environment and evading pulmonary clearance, thereby maintaining UR bacterial homeostasis. These findings corroborate studies by who reported the ability of *Lactobacillus* species to enhance the clearance of *Streptococcus pneumonia* by competitive exclusion; Bertuccini, et al. who also showed the inhibitory activities of Lactobacillus species to *Staphylococcus aureus* and *E. coli* in the human vagina; and Timsit, et al., who reported also that in cattle selected strains of *lactobacillus* administered intranasally resulted in upper respiratory resistance to colonization *by M. haemolytica*.

For lung morphometry, mild changes were observed in the iLp treated groups (A-iLp 3x; B- iLp 5x; and C-iLp 7x) compared to the control group (D-iNS). This shows that iLp does not produce significant pathological lesions in the lung tissue parenchyma. The presence of mild lesions in experimental animals in the iLp treated group could suggest a previous pathologic condition prior to purchase [17]. The localized effect of iLp on constituents of the Bronchial Lavage Fluid (BALF) showed its ability to increase the Macrophage to Neutrophil (M:N) ratio by 4 folds compared to the normal in this study and by 6 folds as reported in BALF from normal caprine lungs by meaning iLp has the capacity to boost macrophage counts hence, improve local innate immune responses through their highly phagocytic capacity. This finding is also consistent with that of who reported an increased macrophage count in cytological smears from lungs following the intranasal administration of *L. fermentum* in mice. Also, it has been suggested *Lactobacillus* sp., have demonstrated the ability to reduce neutrophilic infiltration following lung injury due to the induction of LPS in Wistar rats [18].

Likewise, a remarkable histopathological change of significance observed in the iLp treated groups was the robust development peribronchial nodules (Bronchus Associated Lymphoid Tissue (BALT)) which were not observed in the control group (D-iNS). The ability of iLp to induce the formation of BALT reflects its capacity to initiate immune response without inflammatory reactions. The higher number and sizes of BALT in group B (iLp 5x) may also increase the antigenic uptake and immunostimulation thereby improving lung immunity.

Conclusion

The findings have shown that intranasal administration of *L. plantarum* poses no serious threat to the respiratory homeostasis of the red sokoto goats because it reduced pathogenic bacterial count in the upper respiratory tract, causes no significant pathological alterations of the pulmonary parenchyma, boosts local as well as systemic immune activities as evidenced by robust BALT formation making it a candidate probiotic of choice in the control of MH induced pneumonia in red sokoto goats. The dosage (2.1×10^9) and duration of administration (5 consecutive days) proved most successful in this study and may be considered for use as a prophylaxis in combination with PPR vaccine for the control of naturally occurring pneumonia.

The effect of *L. plantarum* on clinical indices, haematological parameters and oxidative stress analysis of bronchial Lavage fluid are discussed in a separate article.

Ethical Approval

The study was reviewed and approved by the ethical board of the faculty of veterinary medicine, university of Abuja (UAECAU/ 2020/0028).

Ethical Statement

All the authors of this manuscript have read and approved this article for submission. The content of this document has not been previously published or copyrighted.

Competing Interests

The authors have no competing interests to declare that are relevant to the contents of this article.

Author's Contributions

- TZO wrote the article; analysed and interpreted data regarding gross pathology.
- EBO analysed and interpreted data regarding histopathological findings.
- EGO analysed and interpreted data regarding bacteriological findings.
- TV analysed and interpreted data regarding cytology of the bronchial lavage fluid.
- All the authors of this manuscript have read and approved this article for submission.
- The content of this document has not been previously published or copyrighted.

Acknowledgements

We acknowledge Mr. Alao Omoniyi and the department of microbiology, university of Ibadan for providing us the purified culture of *Lactobacillus plantarum* used in the course of this study

Informed Consent

For this type of study, informed consent is not required.

Consent for Publication

For this type of study, consent for publication is not required.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Monteiro, A., et al. "Goat system productions: Advantages and disadvantages to the animal, environment and farmer." *Goat Sci.* (2017):351-366.
2. Bourn, D., et al. "Nigerian livestock resources survey." *Wld Anim Rev* 78.1 (1994):49-58.
3. Adeyemi, M.T., et al. "Interactions between gastrointestinal parasitism and pneumonia in Nigerian goats." *J Parasit Dis.* 41 (2017):726-733.
4. Tenuche, O.Z., et al. "Prevalence of ginger, age and breed in the occurrence of pneumonia in goats slaughtered at gwagwalada abbatoir, Abuja." *J Agr And Vet Sci.* 12.9 (2019):72-77.
5. Jarikre, T. A., et al. "Pattern and associated risk factors of caprine pneumonia complex in Nigeria." *Asian Pac J Trop Dis.* 6.3 (2016): 179-183.
6. Okwelum, O.O., et al. "Prevalence of suspected peste des petits ruminants infection and complicating bacteria in goats in Abeokuta, Ogun State, Nigeria." *Niger J Anim Prod*. 44.3 (2017):43-48.
7. Holman, D.B., et al. "Injectable antimicrobials in commercial feedlot cattle and their effect on the nasopharyngeal microbiota and antimicrobial resistance." *Vet Microbiol.* 214 (2018):140-147.
8. Holman, D.B., et al." Antibiotic treatment in feedlot cattle: A longitudinal study of the effect of oxytetracycline and tulathromycin on the fecal and nasopharyngeal microbiota." *Microbiome.* 7.1 (2019):1-14.
9. Emikpe, B.O., et al. "Intranasal inactivated recombinant *Mannheimia hemolytica vaccine is not protective against naturally occurring pneumonia in Nigerian Goats*." *Afr J Biomed Res.* 16.3 (2013):185-191.
10. Rice, J.A., et al. "Mannheimia haemolytica and bovine respiratory disease." *Anim Health Res Rev.* 8.2 (2007):117-128.
11. Hill, C., et al. "The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic." *Nat Rev Gastroenterol Hepatol.* 11.8 (2014): 506-514.
12. Van, H., et al. "Identification of *Lactobacillus plantarum genes modulating the cytokine response of human peripheral blood mononuclear cells*." *BMC microbiology.* (2010):1-13.
13. Dowarah, R., et al. "The use of Lactobacillus as an alternative of antibiotic growth promoters in pigs: A review." *Anim Nutr.* 3.1 (2017): 1-6.
14. Sirichokchatchawan, W., et al. "Protective effects of cell free supernatant and live lactic acid bacteria isolated from Thai pigs against a pandemic strain of porcine epidemic diarrhea virus." *Probiotics Antimicrob Proteins.* 10 (2018):383-390.
15. Budden, K.F., et al. "Emerging pathogenic links between microbiota and the gut-lung axis." *Nat Rev Microbiol.* 15.1 (2017):55-63.
16. Timsit, E., et al. "Respiratory bacterial microbiota in cattle: From development to modulation to enhance respiratory health." *Vet Clin North Am Food Anim Pract.* 36.2 (2020): 297-320.
17. Ezeasor, C., et al. "Intranasal Peste des petits ruminants virus vaccination of goats using *Irvingia gabonensis.* gum as delivery system: hematological and humoral immune responses." J *Immunoass Immunochem.* 42.1 (2021):82-94.
18. Stevenson, B. "Common bacterial culture techniques and media." *Curr Protoc Microbiol.* (2006): A-4A.

Cite this article: Oremeyi TZ, et al. "Effects of *Lactobacillus plantarum* Administration in Peste Des Petits Ruminants Vaccinated Red Sokoto Goats: Changes in Nasal Bacterial Flora, Bronchoalveolar Cells and Lung Pathology". J Microbiol Immunol, 2023, 5(1), 1-4.